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(uemura or okui or kominami or yamaguchi or mitsui).in. and (vecto\$3 or plasmid\$3) and
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1

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L2

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<u>L1</u>	secret\$5 same tag\$3 same (vecto\$3 or plasmi\$3) same cleav\$5 same space\$3	6	<u>L1</u>

END OF SEARCH HISTORY

WEST**Generate Collection****Print****Search Results - Record(s) 1 through 6 of 6 returned.****1. Document ID: US 20030166160 A1**

L1: Entry 1 of 6

File: PGPB

Sep 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030166160

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030166160 A1

TITLE: Compounds and molecular complexes comprising multiple binding regions directed to transcytotic ligands

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hawley, Stephen B.	San Diego	CA	US	
Chapin, Steven	San Diego	CA	US	
Sheridan, Philip L.	San Diego	CA	US	
Houston, L. L.	Del Mar	CA	US	
Glynn, Jacqueline M.	San Diego	CA	US	

US-CL-CURRENT: 435/69.7; 435/320.1, 435/325, 435/6, 530/350, 536/23.5

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc
Image												

2. Document ID: US 20030161809 A1

L1: Entry 2 of 6

File: PGPB

Aug 28, 2003

PGPUB-DOCUMENT-NUMBER: 20030161809

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030161809 A1

TITLE: Compositions and methods for the transport of biologically active agents across cellular barriers

PUBLICATION-DATE: August 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Houston, L. L.	Del Mar	CA	US	
Sheridan, Philip J.	San Diego	CA	US	
Hawley, Stephen B.	San Diego	CA	US	
Glynn, Jacqueline M.	San Diego	CA	US	
Chapin, Steven	San Diego	CA	US	

US-CL-CURRENT: 424/85.2; 424/178.1, 435/6, 514/44, 530/351, 530/391.1, 530/395

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc
Image												

3. Document ID: US 20020160482 A1

L1: Entry 3 of 6

File: PGPB

Oct 31, 2002

PGPUB-DOCUMENT-NUMBER: 20020160482

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020160482 A1

TITLE: Methods for protein purification

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Abrahmsen, Lars	Bromma		SE	
Nilsson, Joakim	Danderyd		SE	

US-CL-CURRENT: 435/191; 435/320.1, 435/325, 435/69.1, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc
Image												

4. Document ID: US 20020009455 A1

L1: Entry 4 of 6

File: PGPB

Jan 24, 2002

PGPUB-DOCUMENT-NUMBER: 20020009455

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020009455 A1

TITLE: DNA encoding a novel PROST 03 polypeptide

PUBLICATION-DATE: January 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Lau, Ted	Alameda	CA	US	
Lin, Richard J.	Danville	CA	US	
Parkes, Deborah	Hayward	CA	US	
Parry, Gordon	Walnut Creek	CA	US	
Schneider, Douglas W.	Lafayette	CA	US	
Steinbrecher, Renate	Walnut Creek	CA	US	
Heuit, Pamela Toy Van	Moraga	CA	US	
Wu, John	Carlisle	MA	US	

US-CL-CURRENT: 424/178.1; 435/325, 435/6, 435/69.1, 435/7.23, 530/350

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC	Draw Desc
Image											

5. Document ID: US 20020004047 A1

L1: Entry 5 of 6

File: PGPB

Jan 10, 2002

PGPUB-DOCUMENT-NUMBER: 20020004047

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020004047 A1

TITLE: DNA encoding a novel RG1 polypeptide

PUBLICATION-DATE: January 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Harkins, Richard	Alameda	CA	US	
Parkes, Deborah	Hayward	CA	US	
Parry, Gordon	Walnut Creek	CA	US	
Schneider, Douglas W.	Lafayette	CA	US	
Steinbrecher, Renate	Walnut Creek	CA	US	

US-CL-CURRENT: 424/178.1; 435/325, 435/6, 435/69.1, 435/7.23, 530/388.8

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC	Draw Desc
Image											

6. Document ID: US 5935824 A

L1: Entry 6 of 6

File: USPT

Aug 10, 1999

US-PAT-NO: 5935824

DOCUMENT-IDENTIFIER: US 5935824 A

TITLE: Protein expression system

DATE-ISSUED: August 10, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sgarlato; Gregory D.	Los Gatos	CA		

US-CL-CURRENT: 435/69.7; 435/69.8, 530/350, 536/23.4

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC	Draw Desc
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Terms	Documents
secret\$5 same tag\$3 same (vecto\$3 or plasmi\$3) same cleav\$5 same space\$3	6

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WEST**Search Results - Record(s) 1 through 1 of 1 returned.****1. Document ID: EP 1132479 A1**

L2: Entry 1 of 1

File: EPAB

Sep 12, 2001

PUB-NO: EP001132479A1

DOCUMENT-IDENTIFIER: EP 1132479 A1

TITLE: PROTEIN EXPRESSION VECTOR AND UTILIZATION THEREOF

PUBN-DATE: September 12, 2001

INVENTOR-INFORMATION:

NAME	COUNTRY
UEMURA, HIDETOSHI	JP
OKUI, AKIRA	JP
KOMINAMI, KATSUYA	JP
YAMAGUCHI, NOZOMI	JP
IMITSUI, SHINICHI	JP

INT-CL (IPC): C12 N 15/86; C12 N 5/10; C12 P 21/02

EUR-CL (EPC): C12N009/64



Terms	Documents
(uemura or okui or kominami or yamaguchi or mitsui).in. and (vector\$3 or plasmid\$3) and (tag\$3 same cleav\$5 same secret\$5)	1

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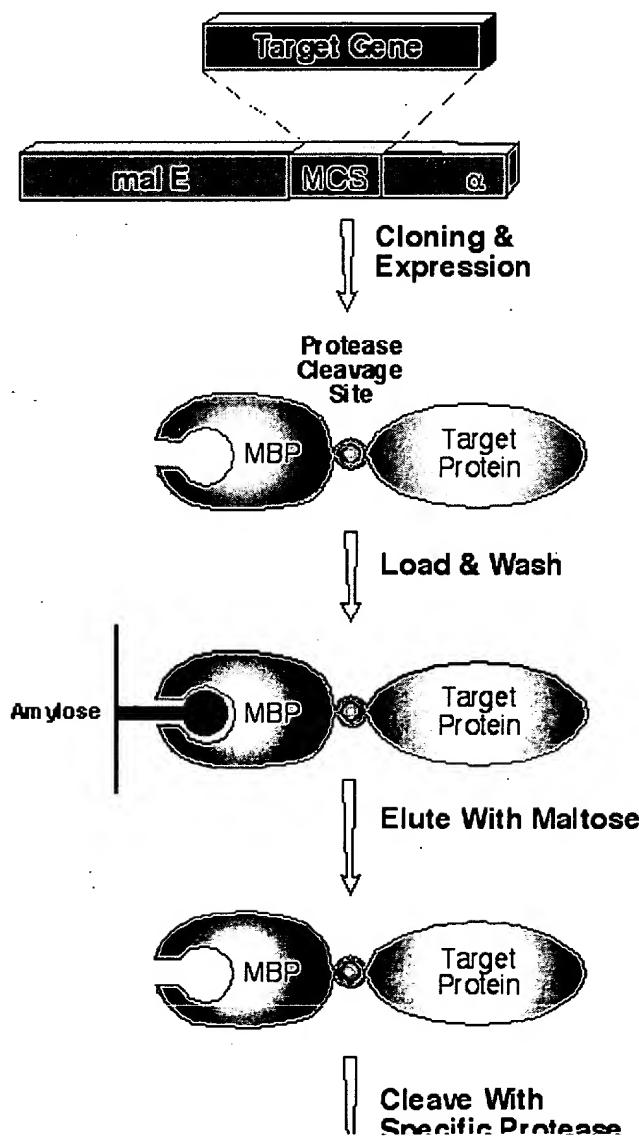
Protein Fusion & Purification (pMALTM) System

#E8000S

\$400 (USA)

Download: [Technical Bulletin](#) and [Manual](#) | [Vector Sequence Files](#) | [FAQs](#) |

Description: In the pMALTM Protein Fusion and Purification System, the cloned gene is inserted into a pMAL vector down-stream from the malE gene, which encodes maltose-binding protein (MBP). This results in the expression of an MBP-fusion protein (1,2,3). The technique uses the strong P_{tac} promoter and the translation initiation signals of MBP to express large amounts of the fusion protein. The fusion protein is then purified by a one-step affinity purification specific for MBP (Figure 1) (4).



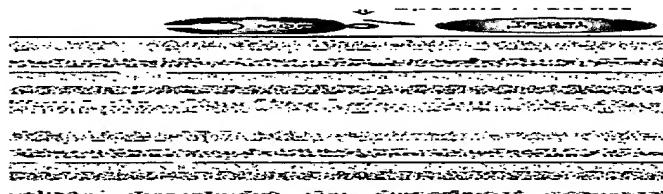


Figure 1: Schematic of the pMAL System.

The system uses the pMAL vectors which are designed so that insertion interrupts a *lacZa* gene allowing a blue-to-white screen for inserts on X-gal (5). The vectors include a sequence coding for the recognition site of a specific protease. This allows the protein of interest to be cleaved from MBP after purification, without adding any vector-derived residues to the protein (6). For this purpose, the polylinker includes a restriction site superimposed on the sequence coding for the site of the specific protease. This is where the gene of interest is inserted.

Expression from the pMAL vectors yields up to 100 mg fusion protein from a liter of culture (Figure 2). In most cases, the expressed protein is soluble, as fusion to MBP has been proven to enhance the solubility of proteins expressed in *E. coli* (7). While no expression system works with every cloned gene, the pMAL Protein Fusion and Purification System gives substantial yields of protein in more than 75% of the cases tested. A chapter in *Current Protocols in Molecular Biology* (3) provides an in-depth analysis of the use of the pMAL vectors.

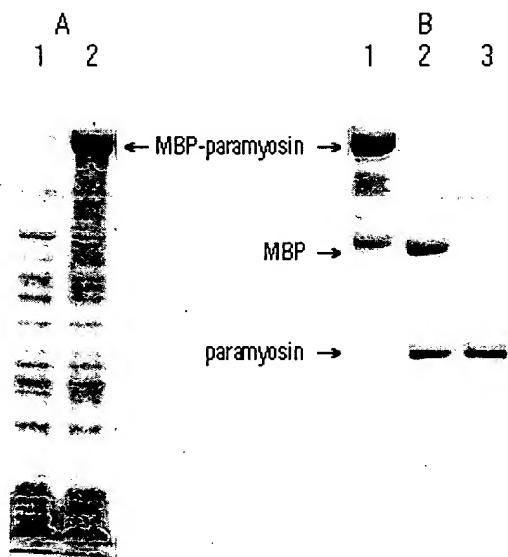


Figure 2: SDS-polyacrylamide gel electrophoresis of fractions from the purification of MBP-paramyosin-Sal.

A: Lane 1: uninduced cells. Lane 2: induced cells.

B: Lane 1: purified protein eluted from amylose column with maltose. Lane 2: purified protein after Factor Xa cleavage. Lane 3: paramyosin fragment eluted from second amylose column.

The System Includes:

- pMAL-c2X: 10 µg

- pMAL-p2X: 10 µg
- Amylose Resin: 15 ml (binding capacity ~40 mg)
- Factor Xa: 50 µg
- Anti-MBP antiserum: 25 µl (for Western blot analysis)
- MBP2*: 10 µg (marker for SDS-polyacrylamide gels)
- MBP2*-paramyosin-DSal: 100 µg (control for Factor Xa cleavage)
- *E. coli* Host
- Comprehensive Instruction Manual

Advantages of the pMAL System:

- Reliable expression: substantial yields (up to 100 mg/L) in more than 75% of the cases tested.
- Expression in either the cytoplasm or periplasm: periplasmic expression enhances folding of proteins with disulfide bonds.
- Fusion to MBP has been shown to enhance the solubility of proteins expressed in *E. coli* (7).
- Gentle elution with maltose: no detergents or harsh denaturants.

References:

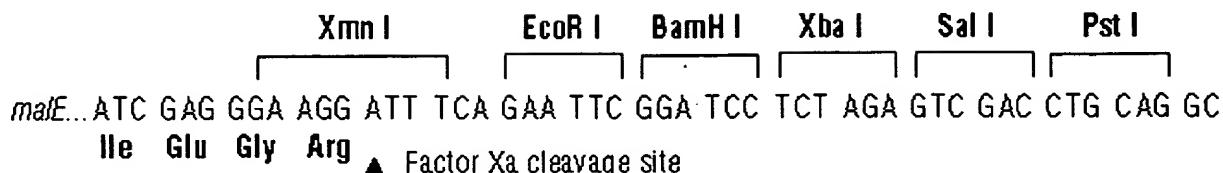
1. Guan, C. et al. (1987) *Gene* 67, 21–30.
2. Maina, C. V. et al. (1988) *Gene* 74, 365–373.
3. Riggs, P. D. (1990). In F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl (Eds.), *Current Protocols in Molecular Biology* (pp. 16.6.1–16.6.10). New York: John Wiley & Sons, Inc.
4. Kellerman, O. K. and Ferenci, T. (1982). In W. A. Wood (Ed.), *Methods in Enzymology* Vol. 90, (pp. 459–463). New York: Academic Press.
5. Yanisch-Perron, C. et al. (1985) *Gene* 33, 103–119.
6. LaVallie, E. R. and McCoy, J. M. (1990). In F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl (Eds.), *Current Protocols in Molecular Biology* (pp. 16.4.1–16.4.17). New York: John Wiley & Sons, Inc.
7. Kapust and Waugh (1999) *Protein Science* 8, 1668–1674.

pMAL™ Companion Products

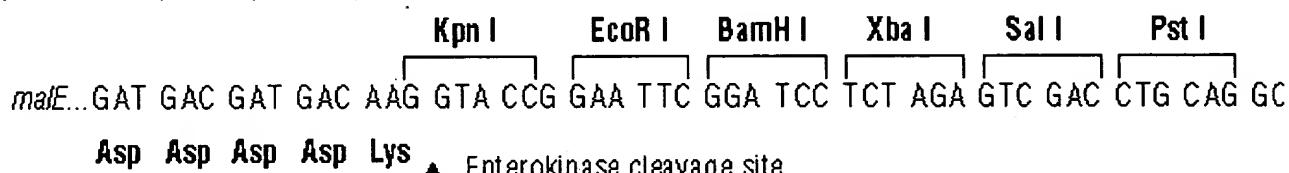
Vectors:

Description: The pMAL™-c2 series of vectors have an exact deletion of the *malE* signal sequence, resulting in cytoplasmic expression of the fusion protein. The pMAL-p2 series of vectors contain the normal *malE* signal sequence, which directs the fusion protein through the cytoplasmic membrane. All of the vectors include a sequence coding for the recognition site of a specific protease [Factor Xa (X), Enterokinase (E) or Genenase™ I (G)] which allows the protein of interest to be cleaved from MBP after purification.

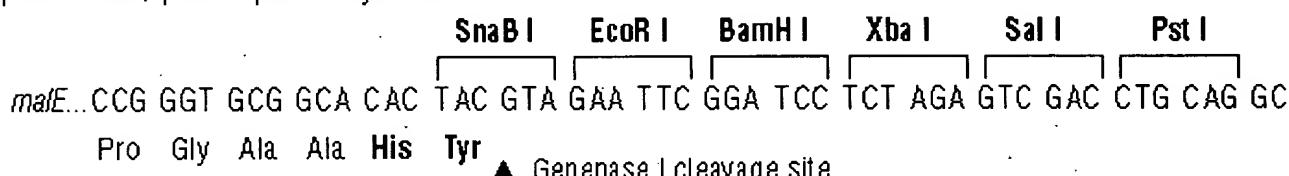
pMAL-c2X, pMAL-p2X Polylinker



pMAL-c2E, pMAL-p2E Polylinker



pMAL-c2G, pMAL-p2G Polylinker



N8076S	<u>pMAL-c2X</u>	10 µg	\$75 (USA)
N8077S	<u>pMAL-p2X</u>	10 µg	\$75 (USA)
N8066S	<u>pMAL-c2E</u>	10 µg	\$75 (USA)
N8067S	<u>pMAL-p2E</u>	10 µg	\$75 (USA)
N8068S	<u>pMAL-c2G</u>	10 µg	\$75 (USA)
N8069S	<u>pMAL-p2G</u>	10 µg	\$75 (USA)

Note: *pMAL-c2X* and *pMAL-p2X* are supplied with the *pMAL Protein Fusion and Purification System* (NEB #E8000S). Others are not supplied with the system.

Amylose resin:

Description: Affinity matrix used for isolation of proteins fused to maltose-binding protein. It is a composite amylose/agarose bead.

Binding Capacity: 2–4 mg MBP/ml bed volume.

Shipping: Supplied pre-swollen in 20% ethanol. Store at 4°C.

E8021S	15 ml	\$100 (USA)
E8021L	100 ml	\$300 (USA)

Factor Xa, Enterokinase and Genenase™ I :**Factor Xa**

E8010S	50 µg	\$50 (USA)
E8010L	250 µg.	\$200 (USA)

Enterokinase (not supplied with the system)

E8070S	5 units	\$80 (USA)
E8070L	25 units	\$320 (USA)

Genenase™ I (not supplied with the system)

E8075S	50 µg	\$50 (USA)
E8075L	250 µg	\$200 (USA)

Anti-MBP serum:

Description: Rabbit antiserum prepared by immunizing with affinity-purified maltose-binding protein.

Concentration and Shipping: End-point dilution titer by Elisa greater than 10–4. Supplied frozen. Store at –20°C.

E8030S	0.2 ml	\$100 (USA)
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Anti-MBP Monoclonal Antibody: (not supplied with the system)

Description: Anti-MBP Monoclonal Antibody is purified from tissue culture fluid of hybridoma cell line B48 by protein A affinity chromatography

Concentration and Shipping: 1 mg/ml. Supplied in 10 mM HEPES (pH 7.5), 150 mM NaCl and 50% glycerol. Store at –20°C.

E8032S	0.05 ml	\$125 (USA)
E8032L	0.25 ml	\$500 (USA)

MBP2*-paramyosin DSal:

Description: MBP2*-paramyosin-DSal is a maltose binding fusion protein (MBP) containing a Factor Xa site between the MBP and paramyosin domains.

Molecular Weight: 70.2 kDa.

Concentration and Shipping: 5 mg/ml. Supplied in 20 mM Tris-HCl (pH 7.2), 0.2 M NaCl, 10 mM b-mercaptoethanol, 50% glycerol and 1 mM sodium azide. Store at .20°C.

E8051S	100 µg	\$50 (USA)
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MBP2* Protein:

Description: MBP2* is wild type MBP plus the amino acids encoded by the pMAL-c2 polylinker. It is purified from cells bearing a derivative of pMAL-c2 with a linker containing a stop codon inserted in the Xmn I site.

Molecular Weight: 42.5 kDa.

Concentration and Shipping: 4–8 mg/ml. Supplied in 20 mM Tris-HCl (pH 7.2), 0.2 M NaCl, 1 mM EDTA, and 50% glycerol. Store at –20°C.

E8044S	1.0 mg	\$50 (USA)
E8044L	5.0 mg	\$200 (USA)

malE and M13/pUC sequencing primer: (not supplied with the system)

malE Primer: Used for sequencing downstream from the malE gene across the polylinker.
5'(dGGTCGTCAGACTGTCGATGAAGCC)3'

M13/pUC sequencing primer: Used for sequencing upstream from the lacZα gene across the polylinker.
5'(dGCCAGGGTTTCCCAGTCACGAC)3'

S1237S	<u>malE Primer</u>	0.5 A ₂₆₀ units	\$95 (USA)
S1224S	<u>M13/pUC Sequencing Primer</u>	0.5 A ₂₆₀ units	\$95 (USA)

M13KO7 Helper Phage: (not supplied with the system)

N0315S M13KO7 Helper Phage 1.8 ml \$55 (USA)

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1: Biotechniques. 1998 Nov;25(5):898-904.

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Positive selection vectors to generate fused genes for the expression of his-tagged proteins.

Van Reeth T, Dreze PL, Szpirer J, Szpirer C, Gabant P.

Universite Libre de Bruxelles, Rhode-Saint-Genese, Belgium.

Epitope tagging simplifies detection, characterization and purification of protein. Gene fusion to combine the coding region of a well-characterized epitope with a coding region for a protein of interest generally requires several subcloning steps. Alternatively, a PCR strategy can be used to generate such a chimeric gene. In addition to its simplicity, this approach allows one to limit the size of the multiple cloning sites present in conventional expression vectors, thus reducing the introduction of artifactual amino-acid sequences into the fused protein. In this communication, we describe new vectors that allow PCR cloning and selection of chimeric genes coding for N- or C-terminal His-tagged proteins. These vectors are based on the control of cell death CcdB direct selection technology and are well adapted to the cloning of blunt-ended PCR products that were generated by using thermostable polymerases that provide proofreading activity.

PMID: 9821593 [PubMed - indexed for MEDLINE]

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1: Anal Biochem. 1999 Apr 10;269(1):10-6.

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ELSEVIER
FULL-TEXT ARTICLE

Influence of the amino acid residue downstream of (Asp)4Lys on enterokinase cleavage of a fusion protein.

Hosfield T, Lu Q.

Stratagene Cloning Systems, Inc., La Jolla, California 92037, USA.
tanya_hosfield@stratagene.com

Related Resources

We have studied the cleavage efficiency of the protease enterokinase (EK) using the novel vector pESP4. pESP4 is a yeast expression vector equipped with ligation-independent cloning sites, a GST purification tag, and a FLAG epitope tag. EK is used to cleave the FLAG and GST tags leaving the protein of interest without any extraneously added amino acids. We have found that EK is relatively permissive of the amino acid residue downstream of the recognition sequence (the P'1 position). This makes EK an ideal choice to use as a protease to cleave any protein of interest cloned within the pESP4 yeast expression vector. Copyright 1999 Academic Press.

PMID: 10094769 [PubMed - indexed for MEDLINE]

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1: J Biotechnol. 1998 Jun 30;62(2):143-51.

Related Articles, Lir

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ELSEVIER
FULL-TEXT ARTICLE

High-level production of human growth hormone in Escherichia coli by a simple recombinant process.

Shin NK, Kim DY, Shin CS, Hong MS, Lee J, Shin HC.

Laboratory of Protein Engineering, Hanhyo Institute of Technology, Yusong-ku Taejon, South Korea.

Related Resources

Procedures have been devised for producing in *Escherichia coli* high yields of purified recombinant human growth hormone (hGH), by utilizing N-terminal pentapeptide sequence of human tumor necrosis factor-alpha, histidine tag and enterokinase cleavage site as a fusion partner. The fusion protein was produced a soluble protein at the beginning of gene expression, but progressively became insoluble in *Escherichia coli* cytoplasm. The insoluble protein was solubilized by simple alkaline pH shift and purified to near homogeneity by Ni(2+)-chelated affinity chromatography. Following specific enterokinase cleavage, the recombinant hGH was purified by one-step anion exchange chromatography. The ease and speed of this recombinant process, as well as the high productivity, makes it adaptable to the large-scale production of hGH. Moreover, the highly efficient fusion partner could be applied to the production of other therapeutical important proteins.

PMID: 9706704 [PubMed - indexed for MEDLINE]

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L5 23 S L4 AND ENTEROKIN?
L6 12 S L5 AND TRYPSI?
L7 10 S L6 AND (NEUROS? OR KALLIKR?)